

Microwaves, Hyperthermia, and
Human Leukocyte Function

Rochester Univ., NY

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jul 83



U.S. Department of Commerce
National Technical Information Service

NTIS

MICROWAVES, HYPERTHERMIA, AND HUMAN LEUKOCYTE FUNCTION

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R806390 and CR808039

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This study was conducted
in cooperation with the
U.S. Air Force School of Aerospace Medicine and
Aeronautical Systems Division (F33615-81-K-0616), and the
U.S. Air Force Office of Scientific Research (AFOSR-80-0111).

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

TECHNICAL REPORT DATA (Please read instructions on the reverse before completing)		
1. REPORT NO. EPA-600/1-83-008	2.	3. RECIPIENT'S ACCESSION NO. PB8 3 225375
4. TITLE AND SUBTITLE Microwaves, Hyperthermia and Human Leukocyte Function		5. REPORT DATE July 1983
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) N.J. Roberts, S.-T. Lu and M. Michaelson		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Rochester, NY		10. PROGRAM ELEMENT NO. CCGF1A
		11. CONTRACT/GRANT NO. Grant R808039
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Final 8-79 - 6-82
		14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES		
16. ABSTRACT Studies were performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. There were no detectable effects on viability or function of human mononuclear leukocytes resulting from exposure to microwave energy at specific absorption rates up to 4 mW/ml. In contrast to studies in other laboratories, our results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned. This report was submitted in fulfillment of Grant No. R806390 and Cooperative Agreement No. CR808039 by the University of Rochester under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers a period from August 27, 1979 to June 30, 1982, and work was completed as of June 30, 1982.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 22
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

NOTICE

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originated in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

The report that follows describes studies relating to potential effects of exposure to non-ionizing radiation. Human immunological functions were examined after *in vitro* exposure of white blood cells to microwaves, in order to determine whether current standards are adequate.

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ABSTRACT

Studies were performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. There were no detectable effects on viability or function of human mononuclear leukocytes resulting from exposure to microwave energy at specific absorption rates up to 4 mW/ml. In contrast to studies in other laboratories, our results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned.

This report was submitted in fulfillment of Grant No. R806390 and Cooperative Agreement No. CR808039 by the University of Rochester under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers a period from August 27, 1979 to June 30, 1982, and work was completed as of June 30, 1982.

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ACKNOWLEDGEMENTS

We thank M. Bolognino, L. Mraz, R.L. Simons, and N. Lebda--Research Assistants, Department of Medicine, University of Rochester School of Medicine--for skilled technical assistance over the course of these studies.

SECTION 1

INTRODUCTION

All individuals are exposed to radiofrequency/microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves (1-3). These studies were admitted to be poorly reproducible and nonquantitative. Nonetheless, they are frequently cited, and have provided the limited data available on exposure of human leukocytes, to be used by those individuals and agencies that develop environmental health standards (4-6). Many animal systems have been studied, but the species, microwave power intensities, environmental conditions and other factors have been so varied that extrapolation to humans would be exceedingly difficult, even if appropriate (7,8).

The studies reported here were performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. Exposure to microwave energy at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability, or unstimulated or stimulated DNA, RNA, total protein or interferon synthesis by human mononuclear leukocytes. In contrast to the earlier studies cited above, results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Exposure of human mononuclear leukocytes to microwave energy at specific absorption rates up to 4 mW/ml resulted in no detectable effects on cell viability, or unstimulated or stimulated DNA, RNA, total protein, interferon- α or interferon- γ synthesis.

The current studies provide the first clear, reproducible data regarding exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Direct extrapolation to the in vitro setting, with many physiological, homeostatic, integrated systems, is not appropriate. However, these data do suggest that earlier reports of possible microwave effects on human leukocytes, at such energy levels, remain poorly reproducible and should not form a basis for the re-setting of safety standards.

SECTION 3

MATERIALS AND METHODS

CELL SOURCE AND COLLECTION OF BLOOD

Peripheral venous blood was obtained by venipuncture from 21 healthy young adult donors (i.e., 14 male, and 7 female) who were taking no medication at the time of the study. Mononuclear leukocytes were obtained from the heparinized whole blood by Ficoll-Hypaque sedimentation (9). Mononuclear leukocytes obtained by this method consisted of 70% to 80% lymphocytes and 20% to 30% monocytes (10); both cell types are required for optimal responses to mitogens and antigens.

Except as noted in the following, leukocyte cultures were maintained in medium 199 (Gibco, Grand Island, N.Y.) with modified Earle's salts with glutamine, aqueous penicillin G (100 units/ml), and streptomycin (50 μ g/ml). The medium was further supplemented with 10% autologous serum. For studies of total protein synthesis, leukocyte cultures were maintained in leucine-free MEM (Gibco, Grand Island, N.Y.).

EXPOSURE AND SHAM-EXPOSURE TO MICROWAVES

The mononuclear leukocytes were exposed in a waveguide system to 2450 MHz (CW) microwaves at specific absorption rates (SARs) from 0.5 to 4 mW/ml. The waveguide system used in these studies has previously been described in detail (11, 12). Exposure and sham-exposure waveguides are located within a water-jacketed, 37°C CO₂ incubator. Temperature inhomogeneity within the cultures is prevented by continuous shaking of the shelf upon which the waveguides rest. In addition to leukocyte cultures enclosed within waveguides for exposure or sham-exposure, we included control cultures--located within the same incubator but external to the waveguides--which rested upon a stationary shelf. Exposures and sham-exposures were monitored continuously by use of Vitek Electrothermia nonperturbing probes (Vitek, Inc., Boulder, Colo.). No attempt was made to counteract microwave-induced heating of the leukocyte cultures since we wished to observe any potential microwave-induced effects, thermal or otherwise.

The SARs were determined by analysis of steady-state temperature increments, ΔT_{ss} (13). The SAR was the product of: the specific heat (0.97 cal/°C/g); the steady-state temperature increment (°C); and the cooling constant (0.0838/min.). The SARs (in milliwatts per milliliter) in this exposure system could be estimated by the product: $5.67 \times \Delta T_{ss}$. During prolonged exposures, changes in the thermal environment were expected. The relation between the SAR and the steady-state temperature increment was best represented by a

constant (4.63), determined empirically by use of culture medium exposed (absorbed) between 5 and 45 mW/ml.

The change in steady-state temperature (mean $\pm$ S.E.) of cultures exposed to microwaves at respective SARs of 0.5, 1.0, and 4 mW/ml were 0.10 ± 0.03 , 0.25 ± 0.03 , and $0.89^\circ\text{C} \pm 0.04$. The final culture temperatures (mean $\pm$ S.E.) were respectively 37.67 ± 0.13 , 37.72 ± 0.15 , and $38.40^\circ\text{C} \pm 0.08$.

ASSAYS OF LEUKOCYTE VIABILITY

Leukocyte viability was determined, from 1 to 7 days after exposure or sham-exposure to microwaves, by use of total cell counts and assays for percent of cells able to exclude trypan blue dye or ethidium bromide (10). The assays of leukocyte function (described in the following) provided additional and even more substantial evidence of leukocyte viability. For example, mitogen-stimulated lymphocyte transformation responses of the human mononuclear leukocytes requires participation of both viable monocytes and viable lymphocytes (10), as does production of PHA-induced interferon- γ (14). Interferon- α is actively produced by viable monocytes after exposure to influenza virus, and preformed interferon is not released by killed cells (14,15).

ASSAYS OF DNA, RNA, AND TOTAL PROTEIN SYNTHESIS

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis by the mononuclear leukocytes were assayed, using established methods, by cellular incorporation of the tritiated precursors thymidine, uridine, and leucine, respectively (10, 16-19). In brief, mononuclear leukocytes were added to quadruplicate wells of sterile microtiter plates (Costar, Cambridge, Mass.) at a concentration of 5×10^5 cells/ml (1×10^5 cells per well). To the cell cultures was added medium alone, or medium containing phytohemagglutinin (PHA)-M (Difco, Detroit, Mich.). The final volume of the cultures was 0.2 ml per well. PHA was added at an optimal concentration of 160 $\mu\text{g/ml}$, shown to yield maximum lymphocyte transformation responses with control mononuclear leukocytes (10,16), and at several suboptimal concentrations (80, 40, 20, and 10 $\mu\text{g/ml}$). Microtiter plates were then incubated at 37°C in 5% CO_2 and air. Cultures were pulsed with the tritiated precursor for the terminal 5 hr of incubation, and were harvested with a semiautomatic cell harvester (Brandel, Inc., Gaithersburg, Md.). Samples were counted with a liquid scintillation counter. Cells were pulsed and harvested from immediately ("zero" days) to 5 days after exposure or sham-exposure to microwaves. For each individual experiment, arithmetic mean counts per minute (cpm) of quadruplicate cultures were determined.

The absolute counts per minute of tritiated precursor incorporated by the normal (control) PHA-stimulated cells of different individuals varied, as previously established (16). Within each individual experiment, however, the relative responses of microwave-exposed, sham-exposed, and control mononuclear leukocytes were consistent.

ASSAYS OF INTERFERON PRODUCTION

The interferon activity in supernatant culture fluids of unstimulated, influenza virus-stimulated, and PHA-stimulated mononuclear leukocytes was assayed by the inhibition of plaque formation by vesicular stomatitis virus in human foreskin fibroblast cultures, as previously described (14,15). Production of interferon- α was induced by exposure of the leukocytes to influenza A/AA/Marton/43 HON1 at multiplicities of infection (MOI) of 10, 1.0, and 0.1. Production of interferon- γ was induced by exposure of the leukocytes to PHA at a concentration of 160 μ g/ml. The minimum interferon titer detected in these studies was 5 units/ml. Results are presented in mean $\log_2$ units/ml $\pm$ S.E.

STATISTICAL ANALYSES

Analysis of variance and Student's t -test were selected for analysis of the data.

SECTION 4

RESULTS

LEUKOCYTE VIABILITY

Exposure of the leukocytes at an SAR of 4 mW/ml produced no significant changes in cell viability for up to one week after exposure (Table 1). Results were similar with exposures at lower SARs.

TABLE 1. TOTAL VIABLE MONONUCLEAR LEUKOCYTES AFTER EXPOSURE TO MICROWAVE ENERGY AT SAR = 4 mW/ml

Exposure	Days of Exposure ^a					
	1	2	4	5	6	7
Microwave	58±7 ^b	60±9	41±4	47±16	39±10	40±10
Sham	65±11	63±15	39±2	72±36	41±9	37±8
Control	54±6	56±6	41±8	46±13	36±7	38±11

^aInsufficient numbers of observations (<5) were available 3 days after exposure. Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

^bData represent mean total number of viable cells (total cells X percent viable X 10⁻⁴, ± S.E.

DNA, RNA, AND TOTAL PROTEIN SYNTHESIS

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis were examined after exposure of the mononuclear leukocytes to microwaves at SARs of 4 mW/ml or less. No significant differences existed between microwave-exposed (4 mW/ml), sham-exposed, and control leukocytes in unstimulated DNA, RNA, or protein synthesis (Figures 1-3, respectively), or in responses of the leukocytes to an optimal concentration of mitogen (Figures 1-3). Results were similar using suboptimal concentrations of mitogen, and using lower SARs (0.5 and 1.0 mW/ml) for the microwave-exposed

cultures (data not shown). Microscopic inspection of Wright-Giemsa-stained cytospin preparations did not reveal any discrepancies between morphologic lymphocyte blastogenesis (used in some of the studies cited earlier) and determinations using incorporation of the radiolabelled precursors.

INTERFERON SYNTHESIS

In addition to our determinations of total protein synthesis, we measured spontaneous production of interferon (none detected in any cultures), as well as production of influenza virus-induced interferon- α and PHA-induced interferon- γ , at 1 and 3 days after induction (14, 15). By 24 hr, virtually all detectable virus-induced interferon- α was present, with equivalent amounts being produced by microwave-exposed (SAR = 4 mW/ml), sham-exposed, and control leukocytes (Figure 4A). Equivalent interferon titers were produced by the leukocytes whether influenza virus was added at a MOI of 10 (Figure 4A), or at an MOI of 1.0 or 0.1 (data not shown). PHA-induced interferon- γ , usually produced by 48-72 hr, was not detected in any culture supernatant fluid at 24 hr. By 72 hr, interferon- γ was detected in all PHA-induced cultures, with no significant differences appearing between the microwave-exposed (SAR = 4 mW/ml), sham-exposed, and control leukocytes (Figure 4B). Results were similar with exposures to microwaves at lower SARs.

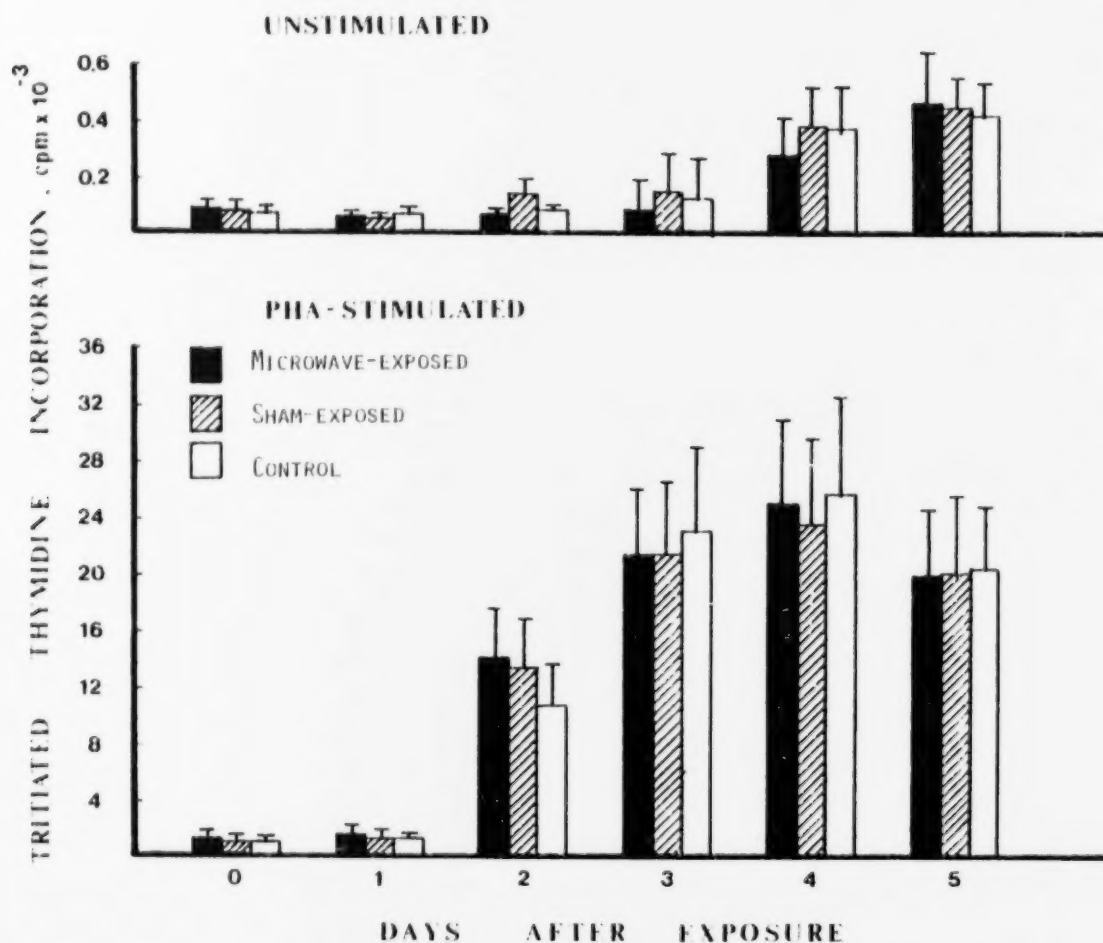


Figure 1. DNA synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes.

SAR = 4 mW/ml. DNA synthesis by unstimulated cells and by cells stimulated by a optimal concentration of PHA (160 μ g/ml) is shown. Columns indicate mean counts per minute tritiated thymidine incorporated $\pm$ S.E., from immediately to 5 days after exposure.

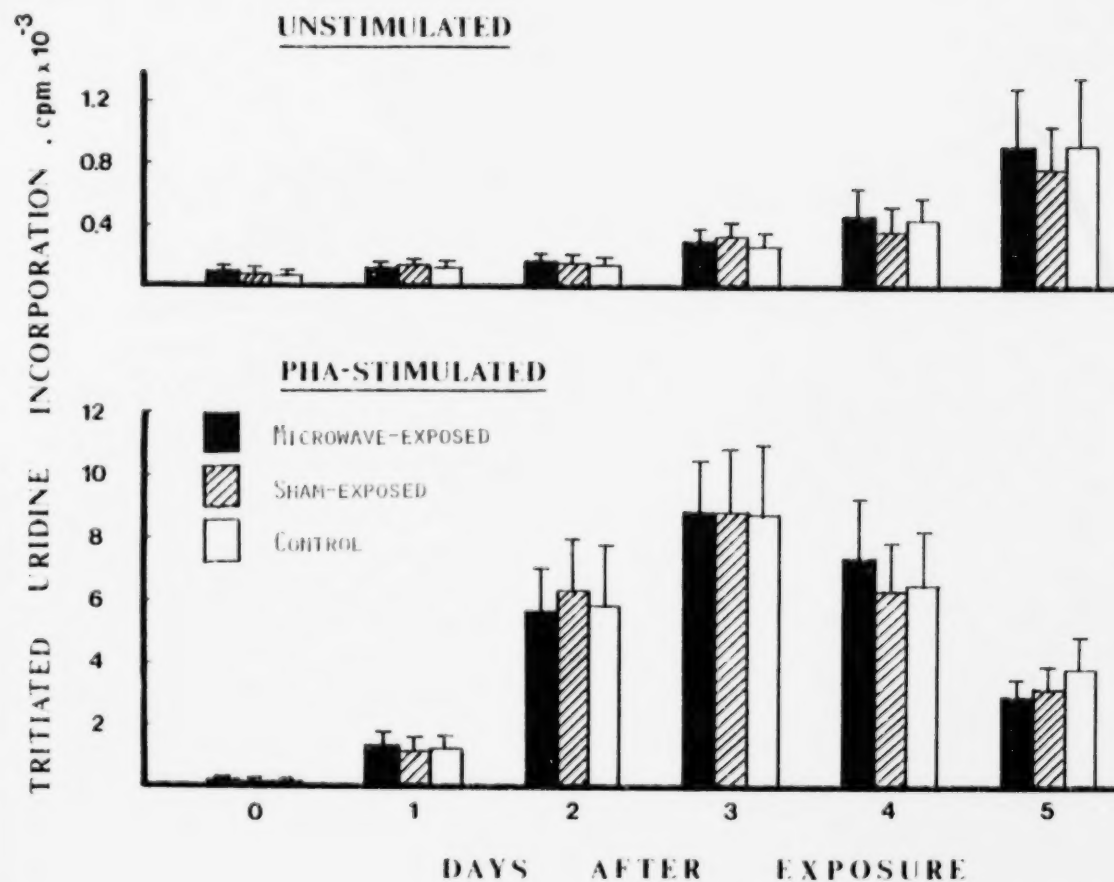


Figure 2. RNA synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes.

SAR = 4 mW/ml. RNA synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160 μ g/ml) is shown. Columns indicate mean counts per minute tritiated uridine incorporated $\pm$ S.E., from immediately to 5 days after exposure.

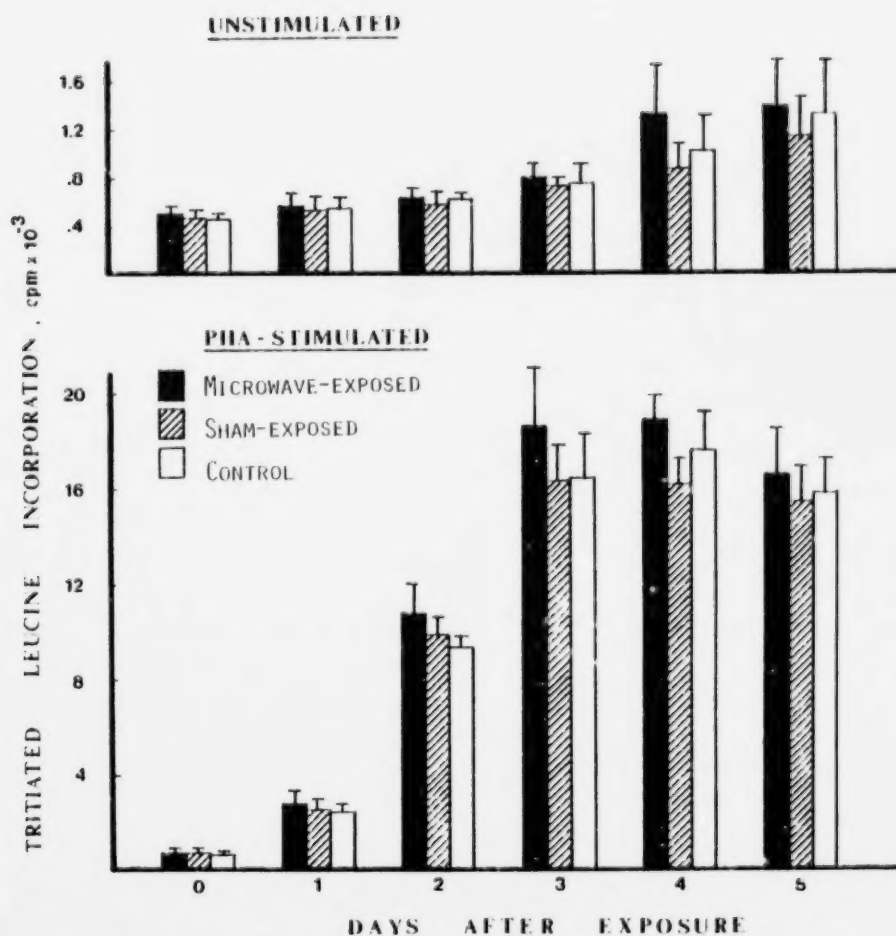


Figure 3. Protein synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes.

SAR = 4 mW/ml. Protein synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160 μ g/ml) is shown. Columns indicate mean counts per minute tritiated leucine incorporated $\pm$ S.E., from immediately to 5 days after exposure.

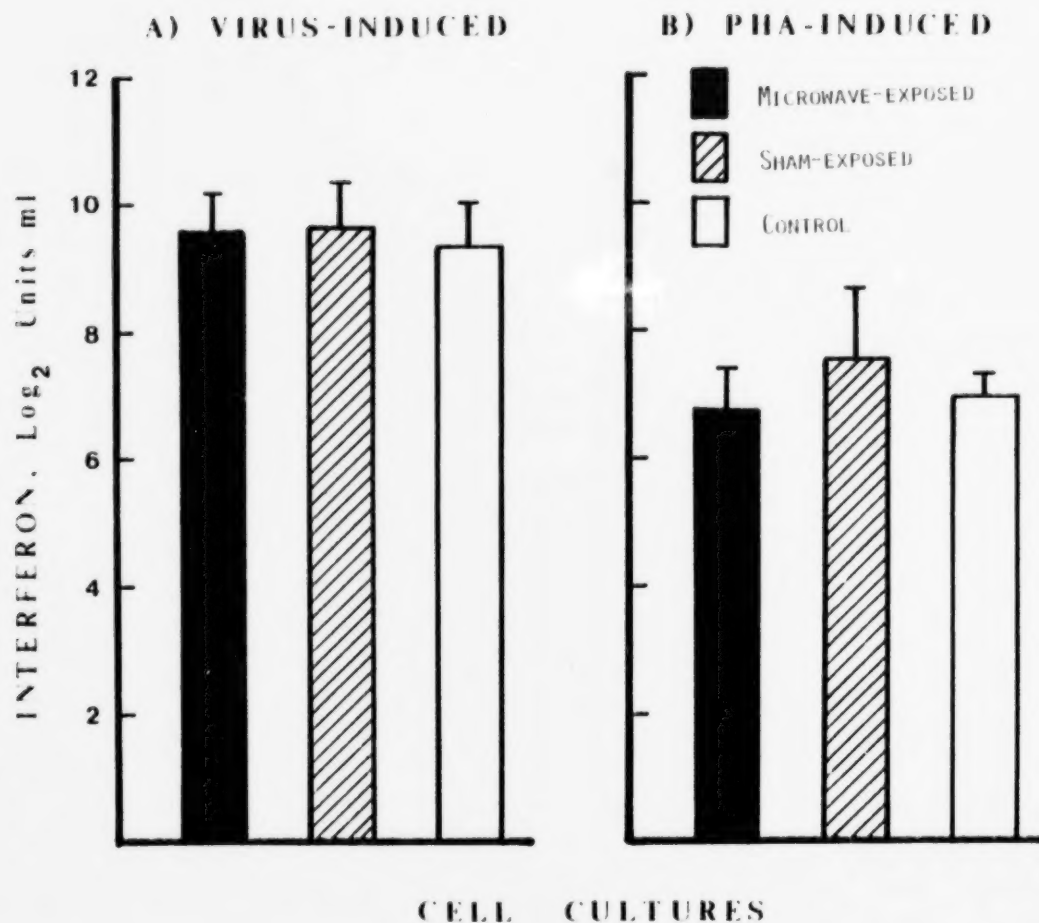


Figure 4. Interferon- α and interferon- γ synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes.

SAR = 4mW/ml. (A) Interferon- α titers present 24 hr after induction with influenza virus; and (B) interferon- γ titers present 72 hr after incubation with PHA. Columns indicate mean log₂ units/ml $\pm$ S.E.

SECTION 5

DISCUSSION

The U.S. safety standard limit, proposed by Committee C-95.4 of the American National Standards Institute, is 0.4 mW/g, which is equivalent to 0.4 mW/ml in the cultures of our studies. This standard incorporates at least a 10-fold safety factor relative to bioeffects reported using animal models. In the current studies, exposure of human mononuclear leukocytes to microwave energy at SARs up to 4 mW/ml resulted in no detectable effects on cell viability, or unstimulated or stimulated DNA, RNA, total protein, interferon- α , or interferon- γ synthesis.

Our current studies provide the first clear and reproducible data concerning exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Direct extrapolation to the in vivo setting, with many physiological, homeostatic, integrated systems, is not appropriate. However, these data do suggest that earlier reports of possible microwave effects on human leukocytes, at such energy levels, remain poorly reproducible and should not form a basis for the re-setting of safety standards. Most studies of environmental physical factors examine effects on resting cell populations even though, under normal conditions, man is commonly exposed to more than one environmental stress at a time (20). Thus, our results are notable further for indicating that human leukocytes exposed to microwaves, as a potential physical stress factor, can respond normally to a second biological factor, such as the commonly encountered infectious agent, influenza virus.

The current studies do not exclude the existence of microwave-induced effects on human leukocytes resulting from exposures at greater SARs. Such exposures commonly produce effects that can be related to the degree and/or the rate of heating of the cell cultures or tissues in vivo (7,21). Furthermore, the current studies do not completely exclude potential microwave-induced effects resulting from exposure at similar SARs but applied by almost innumerable different possible wave forms (e.g., frequencies, modulations, etc.).

The ubiquitous distribution of microwave energy and the potential differences between animal models and humans suggest that further investigations with human leukocytes and other cells may be warranted. The literature regarding microwaves includes animal studies reporting deleterious effects attributed to exposure as well as animal studies reporting beneficial effects, over a broad range of SARs. Fever or moderate hyperthermia, including microwave-induced hyperthermia, has been shown to enhance human leukocyte and animal leukocyte functions, and has been shown to enhance

survival in animals challenged with bacteria, viruses, or tumors (7,8,17,18, 22,23). Thus, further investigations regarding microwave exposure should not only serve to define and limit health hazards for humans, but also to define and expand potential health benefits, such as the use of microwave-induced hyperthermia in the treatment of cancer.

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